1,25-Dihydroxyvitamin D Production Stimulated by Dibutyryl 3', 5'-Cyclic AMP in Normal Subjects and a Patient with Pseudohypoparathyroidism

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UNAKAMI, H., FURUKAWA, Y., SOHN, H.E., YUMITA, S., MIURA, R., SASAKI, A., KOKUBUN, M., MIURA, Y., YOSHINAGA, K. and NAKANOME, C. *1,25-Dihydroxyvitamin D Production Stimulated by Dibutyryl 3',5'-Cyclic AMP in Normal Subjects and a Patient with Pseudohypoparathyroidism*. Tohoku J. exp. Med., 1982, **138** (4), 411-418 — Dibutyryl 3',5'-cyclic AMP (DBcAMP) was infused in 4 normal subjects and a patient with pseudohypoparathyroidism (PHP) to study its effect on the production of 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$]. In normal subjects, 2.5 mg/kg of DBcAMP increased plasma $1,25(\text{OH})_2\text{D}$, reaching the peak at 6 hr after infusion, while, 6.0 mg/kg of DBcAMP delayed the peak to 12 hr. The maximal increment of plasma $1,25(\text{OH})_2\text{D}$ was 19.7 ± 2.8 pg/ml (mean $\pm$ s.d.) in normals with 6.0 mg/kg of DBcAMP and 12.2 ± 1.0 pg/ml (mean $\pm$ s.d.) with 2.5 mg/kg, respectively. There was a significant difference between the doses ($p < 0.01$). Plasma 24,25-dihydroxyvitamin D [$24,25(\text{OH})_2\text{D}$] showed no significant change after DBcAMP infusion in normal subjects. In a patients with PHP, however, 2.5 mg/kg of DBcAMP markedly increased plasma $1,25(\text{OH})_2\text{D}$ to reach the peak (48.9 pg/ml) at 14 hr and plasma $24,25(\text{OH})_2\text{D}$ was decreased reciprocally. After DBcAMP infusion, depression of serum P, slight decrease in %TRP and the elevation of IRI were proved in normal subjects, while in a patient with PHP, there was a marked depression of %TRP. It is suggested that the increments of plasma $1,25(\text{OH})_2\text{D}$ by infusion of DBcAMP is dose-dependent in normal subjects, and that the $1,25(\text{OH})_2\text{D}$ productivity by DBcAMP may be accelerated in a patient with PHP. ——— dibutyryl 3',5'-cyclic AMP (DBcAMP); 1,25-dihydroxyvitamin D [$1,25(\text{OH})_2\text{D}$]; 24,25-dihydroxyvitamin D [$24,25(\text{OH})_2\text{D}$]; pseudohypoparathyroidism (PHP)

Parathyroid hormone (PTH) enhances the conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3 in the kidney (Garabedian et al. 1972; Fraser and Kodicek 1973). And some workers suggested that 3',5'-cyclic AMP (cAMP) or DBcAMP reproduced the stimulating effect of PTH on 1,25-dihydroxyvitamin D_3 production by in vitro or in vivo animal experiments (Rasmussen et al. 1972; Larkins et al. 1974; Horiuchi et al. 1977).

Previously we demonstrated that DBcAMP remarkably increased plasma 1,25-

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(OH)₂D in a patient with pseudohypoparathyroidism (PHP) in whom even large doses of exogenous PTH failed to increase plasma 1,25(OH)₂D (Unakami et al. 1982).

In this study, we examined the responsiveness of vitamin D metabolites to DB cAMP infusion in normal subjects and a patient with PHP. In addition, we studied the changes in various hormones and electrolytes which might affect the changes in vitamin D metabolism.

MATERIALS AND METHODS

Four normal subjects (3 males and 1 female, aged 24–39 years) and a patient with PHP were studied.

Case report of a patient with PHP: A 24 year-old man complaining of tetany was admitted to our department for a detailed examination and therapy. He had no somatic signs such as short stature, obesity, brachydactyly and subcutaneous calcification. But laboratory data revealed hypocalcemia (6.4 mg/100 ml), hyperphosphatemia (5.7 mg/100 ml) and high plasma immunoreactive parathyroid hormone (iPTH) concentration (0.60 ng/ml). P clearance was 4.3 ml/min and %TRP was 95.7%, respectively. Moreover, there were no responses of urinary excretion of P and cAMP to 200 units of PTE (Parathormone, Lilly Co. Ltd.). Based on these data, he was diagnosed as PHP type I (Drezner et al. 1973).

After an overnight fast, 2.5 mg/kg of DBcAMP was intravenously infused with 250 ml of normal saline for 1 hr to 3 normal subjects and a patient with PHP. Blood was drawn before and 30 min, 1, 2, 3, 6, 12 (14) and 24 hr after the infusion for the determination of plasma vitamin D metabolites [25OHD, 1,25(OH)₂D and 24,25(OH)₂D], serum Ca, P, plasma iPTH, calcitonin (CT), growth hormone (GH), prolactin (PRL), catecholamines (CA), immunoreactive glucagon (IRG), immunoreactive insulin (IRI) and blood sugar (BS). Furthermore, hourly urine was collected for 1 hr before and 3 hr after the infusion for the measurement of urinary P. Similarly, 6.0 mg/kg of DBcAMP was intravenously infused with 250 ml of normal saline for 1 hr in 4 normal subjects (1 normal subject was added to the previous 3 normals). Blood and urine were collected as mentioned above.

Plasma vitamin D metabolites were measured according to the method described previously (Unakami et al. 1982). Normal range of each vitamin D metabolite in adult volunteers was: 25OHD, 20.5±5.4 ng/ml (*n*=6, from April to September) or 16.7±5.4 ng/ml (*n*=13, from October to March); 1,25(OH)₂D, 37.2±13.8 pg/ml (*n*=21); 24,25(OH)₂D, 1.73±0.62 ng/ml (*n*=17).

Serum Ca was measured by EGTA titrimetry, serum and urinary P were by Fiske and Subbarow's method (1925). Plasma iPTH, CT, GH, PRL, IRG and IRI were quantitated by the radioimmunoassays, respectively. Plasma CA was measured by the highly sensitive fluorimetry (Miura et al. 1977), and BS was by glucose-oxidase method.

Student's *t* test was used for statistical analyses, and the variance of the mean was expressed as S.D.

RESULTS

In 4 normal subjects, the basal values of plasma vitamin D metabolites were: 25OHD, 14.1±5.5 ng/ml; 1,25(OH)₂D, 39.8±11.8 pg/ml; 24,25(OH)₂D, 2.20±0.82 ng/ml. And those of serum Ca, P and %TRP were: Ca, 9.1±0.3 mg/100 ml; P, 3.4±0.4 mg/100 ml, %TRP, 86.2±3.9%. While, in a patient with PHP, the basal value of plasma 25OHD was 6.5 ng/ml, that of plasma, 1,25(OH)₂D was 12.0 pg/ml and that of plasma 24,25(OH)₂D was 1.11 ng/ml, respectively.

By the infusion of 2.5 mg/kg of DBcAMP, plasma 1,25(OH)₂D increased and reached its peak at 6 hr and then slightly decreased in 3 normal subjects. While, 6.0 mg/kg of DBcAMP increased plasma 1,25(OH)₂D more markedly and the peak

Fig. 1. The time course of the changes in plasma 1,25 (OH)₂D [Δ 1,25(OH)₂D] after DBcAMP infusion in normal subjects with 2.5 mg/kg of DBcAMP ($\circ$ — $\circ$, $n=3$, mean $\pm$ s.d.), in normals with 6.0 mg/kg of DBcAMP ($\square$ — $\square$, $n=4$, mean $\pm$ s.d.) and in a patient with PHP ($\bullet$ — $\bullet$). Hyper-response of Δ 1,25(OH)₂D was seen in a patient with PHP. An arrow indicates the infusion point. * $p<0.05$, † $p<0.01$, ‡ $p<0.005$, § $p<0.001$ by paired t test.

Fig. 2. The time course of the changes in plasma 24,25(OH)₂D [Δ 24,25(OH)₂D] after DBcAMP infusion in normals and a patient with PHP. Each marker represents as in Fig. 1. Marked depression of Δ 24,25(OH)₂D was seen only in a patient with PHP.

was reached at 12 hr after the infusion in 4 normal subjects. In contrast, plasma $1,25(\text{OH})_2\text{D}$ markedly increased to reach the peak at 14 hr by the infusion of 2.5 mg/kg of DBcAMP in a patient with PHP (Fig. 1).

The maximal increment of plasma $1,25(\text{OH})_2\text{D}$ in normals with 6.0 mg/kg of DBcAMP (19.7 ± 2.8 pg/ml) was significantly higher than that of normals with 2.5 mg/kg of DBcAMP (12.2 ± 1.6 pg/ml) ($p < 0.01$). On the other hand, in a patient with PHP, 2.5 mg/kg of DBcAMP brought about a large increment (about 4 times as large as that in normal subjects) of plasma $1,25(\text{OH})_2\text{D}$ which was greater than that in normals with 6.0 mg/kg of DBcAMP.

Plasma $24,25(\text{OH})_2\text{D}$ was not significantly changed in normal subjects. But in a patient with PHP, there was a marked decrement of plasma $24,25(\text{OH})_2\text{D}$ (Fig. 2).

No significant changes were found in plasma 25OHD serum Ca after DBcAMP infusion.

In contrast, serum P and %TRP were significantly depressed at 1 hr and almost restored to the basal level at 3 hr after the infusion in normal subjects. In a patient with PHP, %TRP was markedly decreased, though there was no significant change in serum P (Fig. 3).

In normal subjects, the basal plasma concentrations of the hormones which may affect $1,25(\text{OH})_2\text{D}$ production were: iPTH, all lower than 0.40 ng/ml; CT,

Fig. 3. Effect of DBcAMP on serum P and %TRP in normals ($\circ-\circ$, $\Delta-\Delta$ $n=3$, mean $\pm$ s.d.) and a patient with PHP ($\bullet-\bullet$, $\blacktriangle-\blacktriangle$). Marked decrease in %TRP was seen in a patient with PHP. * $p < 0.05$, † $p < 0.01$ by paired t test.

35±15 pg/ml; GH, 3.0±2.2 ng/ml; PRL, 7.3±2.1 ng/ml; epinephrine, 84±15 pg/ml; norepinephrine, 156±56 pg/ml; IRG, 106±49 pg/ml; IRI, 3±2 μU/ml. And that of BS was 92±4 mg/100 ml. Among them, iPTH, CT, GH, PRL and CA

Fig. 4. Effect of DBcAMP on BS (○—○), IRG (Δ—Δ) and IRI (□—□) in 3 normal subjects. Distinct elevation of BS and IRI were seen in contrast with the depression of IRG after DBcAMP infusion. * $p < 0.05$, † $p < 0.005$, § $p < 0.001$ by paired t test.

showed no significant changes after DBcAMP. On the other hand, BS was markedly elevated, reaching the peak at 1 hr and restored to the basal level or further depressed at 2 hr after DBcAMP infusion. Plasma IRG was reciprocally depressed, while plasma IRI was elevated in accordance with the elevation of BS (Fig. 4). In a patient with PHP, the basal value of plasma iPTH was 0.60 ng/ml and that of PRL was 2.3 ng/ml, respectively. There were no significant changes in these hormones after DBcAMP infusion.

DISCUSSION

It has been reported by some workers that cAMP or DBcAMP reproduced the stimulating effect of PTH on 1,25(OH)₂D production in in vitro or in vivo animal experiments (Rasmussen et al. 1972; Larkins et al. 1974; Horiuchi et al. 1977). And recently, Yamaoka et al. (1981) reported the same stimulating effect of DBcAMP on 1,25(OH)₂D production in children. In our study, 2.5 mg/kg or 6.0 mg/kg of

DBcAMP distinctly increased plasma $1,25(\text{OH})_2\text{D}$ in 6 to 12 hr after the infusion in normal subjects. Furthermore, there was a significant difference in the response of plasma $1,25(\text{OH})_2\text{D}$ between the dose of 2.5 mg/kg and 6.0 mg/kg of DBcAMP ($p < 0.01$), indicating that the increment of plasma $1,25(\text{OH})_2\text{D}$ by DBcAMP is dose-dependent.

PHP type I, as Drezner et al. (1973) distinguished, has the unresponsiveness of cAMP to PTH in the kidney. Some workers reported that the stimulating effect of PTH on $1,25(\text{OH})_2\text{D}$ production, when administered by a bolus, was not seen in this disease (Lambert et al. 1980; Mason et al. 1980). In our previous study, we demonstrated that even large doses of exogenous PTH failed to increase plasma $1,25(\text{OH})_2\text{D}$ in patients with PHP type I. In addition, we revealed that DBcAMP markedly increased plasma $1,25(\text{OH})_2\text{D}$ in a patient with PHP; subsequently we concluded that DBcAMP stimulates 1α -hydroxylase activity and that the process of production of $1,25(\text{OH})_2\text{D}$ after cAMP producing system is not impaired in this disease (Unakami et al. 1982). In this study, we demonstrated the marked increment of plasma $1,25(\text{OH})_2\text{D}$ by DBcAMP infusion in a patient with PHP, which was greater than that in normal subjects. Therefore, the productivity of $1,25(\text{OH})_2\text{D}$ in PHP seems to be accelerated.

Some investigators reported the inhibitory effect of PTH on 24-hydroxylase activity in animal or in vitro experiments (Tanaka et al. 1975; Juan and DeLuca. 1977), but few workers reported the decrement of plasma $24,25(\text{OH})_2\text{D}$ by PTH administration in man. In our previous study, we reported that there were no changes in plasma $24,25(\text{OH})_2\text{D}$ by PTH infusion in normal subjects and patients with PHP, and that DBcAMP markedly decreased plasma $24,25(\text{OH})_2\text{D}$ in a patient with PHP (Unakami et al. 1982). In this study, plasma $24,25(\text{OH})_2\text{D}$ was not changed significantly in normal subjects, but markedly decreased in a patient with PHP. This hyper-response of plasma $24,25(\text{OH})_2\text{D}$ to DBcAMP in a patient with PHP could be accounted for if the same mechanisms as assumed for the hyper-response of plasma $1,25(\text{OH})_2\text{D}$ were applicable to plasma $24,25(\text{OH})_2\text{D}$. But the actual reason for this phenomenon still remains to be elucidated.

By DBcAMP infusion, serum P and % TRP were significantly depressed in normal subjects. In a patient with PHP, greater decrement of %TRP than in normals was observed, though the change in serum P was not significant in this patient. Therefore, the phosphaturic effect caused by DBcAMP seems to be accelerated in patient with PHP. It is well known that hypophosphatemia stimulates 1α -hydroxylase activity, increasing the production of $1,25(\text{OH})_2\text{D}$ (Tanaka and DeLuca 1973). Depression of serum P may possibly have stimulated the production of $1,25(\text{OH})_2\text{D}$ at least in normal subjects.

Recently, it is suggested that in addition to PTH, many hormones such as CT (Horiuchi et al. 1979), GH (Eskildsen et al. 1979; Brown et al. 1980) and PRL (Brown et al. 1980; Bikle et al. 1980) stimulate $1,25(\text{OH})_2\text{D}$ production from 25-OHD. In this study, we followed the changes in various hormones in normal subjects. But there were no significant changes in either these hormones or

plasma iPTH or CA. Therefore, these hormones may not affect the 1,25(OH)₂D production. However, by DBcAMP infusion, BS and plasma IRI were markedly elevated, while plasma IRG was reciprocally depressed. Among these factors, the increment of plasma IRI might increase 1,25(OH)₂D production, since insulin was reported to stimulate the production of plasma 1,25(OH)₂D (Schneider et al. 1977). But the relationship in PHP cannot be discussed here, as no study concerning BS, IRG and IRI were carried out in patient with PHP.

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